

# DISCOVERY

Efficacy of Neem (Azadirachta indica A. Juss) leaf powder on Cowpea Seed bruchid (Callosobruchus maculatus Fab.) during Storage of Cowpea (Vigna unguiculata L. Walp) Seeds

Liamngee K<sup>1</sup>, Fayinminu AO<sup>1</sup>, Iyaji AA<sup>2</sup>, Adegbe AE<sup>1</sup>

<sup>1</sup>Department of Biological Sciences, Benue State University, Makurdi, Nigeria

<sup>2</sup>Centre for Food Technology and Research, Benue State University, Makurdi, Nigeria

## **Article History**

Received: 06 October 2020

Reviewed & Revised: 07/October/2020 to 14/November/2020

Accepted: 15 November 2020 Prepared: 18 November 2020 Published: December 2020

#### Citation

Liamngee K, Fayinminu AO, Iyaji AA, Adegbe AE. Efficacy of Neem (Azadirachta indica A. Juss) leaf powder on Cowpea Seed bruchid (Callosobruchus maculatus Fab.) during Storage of Cowpea (Vigna unquiculata L. Walp) Seeds. Discovery, 2020, 56(300), 832-845

#### **Publication License**



© The Author(s) 2020. Open Access. This article is licensed under a Creative Commons Attribution License 4.0 (CC BY 4.0).

#### **General Note**



Article is recommended to print as color digital version in recycled paper.

# **ABSTRACT**

Efficacy of Neem leaf powder on cowpea seed bruchid (Callosobruchus maculatus Fab.), during storage was investigated. Two groups of cowpea; one free from weevil infestation and the other consisting of infested weevils were obtained from farmers in Makurdi. Neem leaf powders were prepared by drying the leaves of the Neem plant, grinding it with pestle and mortar and sieving to get fine powder; after which three concentrations of 0g, 25g and 50g were prepared for assessment tests. Five pairs each of Callosobruchus maculatus Fab. consisting of males and females were introduced into plastic containers containing 250g each of wholesome white and brown cowpea seeds and left undisturbed for seven days. Thereafter, the weevils were removed and eggs laid were allowed to develop. Emerged progenies were introduced into wholesome brown and white cowpea seeds treated with various concentrations of Neem leaf powder and kept for a period of 28days during which data on quality parameters were taken. The

experiment was arranged in 3x2 factorial in completely randomized design. Days for egg laying in white and brown cowpea were 3 and 5 respectively while that for progeny emergence was 22 and 25days respectively. White cowpea showed significantly higher weight, germination and insect mortality (36.65g, 68.90% and 4.00 respectively) compared to the brown variety (34.73, 60.7 and 3.00). Also significantly higher weight, germination and insect mortality was recorded for cowpea seeds treated with 50g Neem leaf powder (39.44, 79.70 and 5.33 respectively) compared to 10% (36.46, 66.3 and 3.50) and 0% (32.71, 48.30 and 1.67) while higher insect population and grain with holes was recorded for grains treated with 0g of Neem leaf powder (21.17 and 31.33) compared to 25g (19.33 and 20.33) and 50g (15.33 and 15.00) respectively. Neem leaf powder is a potent preservative for cowpea and is therefore a good alternative for chemical preservatives. Adoption of Neem leaf powder should be considered by farmers involved in cowpea storage.

**Keywords:** Cowpea, C. maculatus, Neem leaf powder, Storage.

#### 1. INTRODUCTION

Cowpea [Vigna unguiculata(L.)Walp] also known as blackeye pea, zipper pea, niebe, Southern pea and lobiais one of the most nutritionally important food legumes and a cheap source of protein (25% on a dry weight basis), minerals and vitamins for both urban and rural consumers in developing countries, Southern Europe, Asia, Central and Southern America. Cowpea seeds (dried or fresh), leaves and pods are commonly used as food for humans and a nutritious fodder for livestock. Like other legumes, cowpea contribute to dietary protein instarchy tuber- based diets through their relatively high protein content and to the quality of dietary protein by forming complementary mixtures with staple cereals (Abbey, 2006).

Insect infestation is one of the most important biotic factor affecting cowpea seeds during storage and the cowpea seed bruchid (*Callosobruchus maculatus* Fab.) is the most important storage pest throughout the tropics. In West Africa, up to 100% damage to cowpea may take place in a few months after storage due to *C. maculatus* infestation (Sallam, 2011). About 82% of cowpea grains are produced by small scale farmers and the harvested produce are stored in the farm and other small storage structures (Njonjo *et al.*, 2019). These storage structures coupled with poor storage conditions give rise to quantitative and qualitative losses of stored cowpea grains every year due *C. maculatus* infestation. Quantitative and qualitative assessment of losses in stored cowpea is difficult due to variability in infestation and damage from year to year. Such losses continue to occur due to poor threshing, cleaning, drying and storing techniques used for cowpea production in Africa.

Chemical insecticides such as Actellic dust (powder formulation), Karate (liquid formulation) used to control insect pests have proved to be effective. However, the use of synthetic insecticides in crop protection programs has resulted in lethal effect to nontarget organisms in the agro-ecosystems, pest resurgences and resistance. Also, the expensive nature of some of the available chemicals as well as their toxic effect on human health is also a serious concern (Abulude et al., 2007). As such, biopesticides derived from plants with insecticidal properties are attractive alternatives to chemical insecticides for insect pest management. They are easy to prepare, cheap, in most cases, readily available, non-toxic to humans and have several active ingredients which work synergistically making it difficult for pests to develop resistance (Tello et al., 2013). Hence, the study is set to investigate the efficacy of Neem leaf powder on cowpea seed bruchid of cowpea during storage. The study will provide farmers and crop scientists with substantial information pertaining the effectiveness of Neem plant in cowpea storage. It will aid agricultural research institutes and the government to make policies that will incorporate Neem plant and other botanicals into their brochure of organic preservatives thereby ensuring the dispersal of these knowledge with far reaching impacts. The success in the use of Neem as preservative may serve as impetus for the utilization of indigenous plant based extracts/powders by entrepreneurs for small scale product protection and for possible industrial scale applications. It will further seek to add to the number of available literatures on beneficial impacts of Neem as a preservative in our society, so as to make known to people, the diverse uses of Neem and the multiplicity of functions it can play on crops which have a short shelf life. The study will provide baseline information for researchers and students venturing into the field of postharvest preservation of cowpea.

## 2. MATERIALS AND METHODS

# 2.1. Experimental Location

The study was carried out in the Botany Laboratory of Benue State University, Makurdi. Makurdi is located in North central Nigeria along the Benue River, on latitude 07°43′N and Longitude 08°35′E; it is 104m above sea level and lies in the tropical Guinea-Savannah of West Africa where temperature ranges between 21.7°-24.0°C and a maximum of 29.0°-40°C. It is also an ectone belt that separates the forested South from the true Savannah of North. As such, the vegetation is an assortment of tree and grasses

(Nigerian Metrological Association, 2017). Important cash crops include soybeans, rice, peanuts, mango varieties and Citrus. Other cash crops include palm oil, melon, African pear, chili pepper and tomatoes. Food crops include Yam, Cassava, Sweet potato, Beans, Maize, Millet, Guinea corn and vegetables.

#### 2.2. Collection and preparation of experimental materials

## 2.2.1. Source of cowpea seeds

Cowpea seeds were collected from farmers in Makurdi, Benue State. Two groups of cowpea were obtained for this study. The first group was seeds of two varieties (white and brown) free from physical manifestation of weevil infestation (no holes in grains, eggs of weevils, or visible signs of weevils) while the last group consisted of infested grains with *Callosobruchus maculates* obtained from same location. The infested seeds were stored in plastic containers at room temperature prior to the commencement of laboratory experiment while the uninfected cowpea seeds were sorted and those showing signs of damage in the form of emergent holes, cracks, broken seeds, eggs of weevils and discoloured seeds were discarded. The wholesome seeds were refrigerated at 4-9°C for further studies.

#### 2.2.2. Production of Neem leaf powders

Collection of Neem plant materials were carried out according to the procedure highlighted by Zakki *et al.* (2017). Fresh leaves of *Azadirachta indica* A. Juss (Neem) were obtained from the campus of the Benue State University Makurdi using a knife to cut leaves from the tree. The leaves were put in polythene envelopes and taken to the laboratory for further studies. In the laboratory, the leaves were air-dried for 10 days. The dried leaves were afterwards grounded using a pestle and mortar and sieved to get a fine powder. The powder was stored in well covered clean jars and kept for further studies.

## 2.2.3. Preparation of Neem leaf powder concentrations

Concentrations of the Neem leaf powder applied to 250g of Cowpea were calculated using the formula below.

Powder Concentration (g) = 
$$\frac{Percentage\ Level}{100}$$
 × weight of grains - - - - (i)

Two (2) levels each of 10% and 20% were selected for use in this study. Therefore,

For 10% level,

Powder Concentration (g) = 
$$\frac{10}{100}$$
 x 250.....(ii)

: - Powder concentration (g) = 25g of Neem leaf powder

Weight of grains = 250g

For 15% level,

Powder Concentration = 
$$\frac{20}{100} \times 250$$
 ..... (iii)

= 50g of Neem leaf powder

# 2.3. Experiment I

## Preparation of C. maculatus F. cultures under laboratory conditions for assessment tests (Breeding experiment)

Five pairs each of *Callosobruchus maculates* Fab., consisting of males and females were introduced into containers containing 250g each of wholesome white and brown cowpea seeds respectively. The containers which were plastic, transparent and oval shaped with a flat bottom of about 8-10mm diameter was covered with a 10mm mesh sieve to allow free air circulation and also to prevent insects from escaping. This was carried out at room temperature and relative humidity. The setup was left undisturbed for a period of five to seven days after which the parent weevils were removed and eggs laid by the parent weevils were allowed to develop. Progeny of the weevils in the stored cowpea were afterwards harvested by sieving and then left for another 24 hours ready to be used for quality assessment.

#### 2.4. Experiment II

# Evaluation of the effect of Neem leaf powder on cowpea seed bruchid during storage

Wholesome cowpea seeds (white and brown varieties) previously stored in the fridge were removed and sundried to remove moisture and any resident insects. After that, the cowpea seeds (250g) were placed in plastic containers. Afterwards, each treatment level of Neem leaf powder were added to the seeds in containers and mixed thoroughly to ensure uniform exposure of seeds to Neem leaf powder. Control seeds were left untreated with Neem leaf powder. Thereafter, 5 pairs (males and females) of one day old adult cowpea bruchids were introduced into each set of treated seeds. Each container was covered with a muslin cloth held by a rubber band to allow free air circulation and to prevent insect escape. All these were kept at ambient temperature and examined every 7days for 28 days.

# 2.5. Experimental Design

Factors involved in the experiment

- 3 Rates of application of Neem leaf powder (25g, 50g and 0g)
- 2 varieties of cowpea seeds (white and brown)

Experimental design is a 2  $\times$  3 factorial in a completely randomized design Treatment combinations = 6

Replications = 3

Total number of units = 18

Data collected include:

## Insect population (n)

The total insect population was determined by counting the number of insects in the storage containers.

#### Insect mortality (n)

This was determined by counting the number of insects with no visible characteristic of movement and irritability.

## Grains with holes (n)

Seeds with openings and cracks were counted after visual inspection.

## Seed weight (g)

This was obtained by placing 100 seeds of cowpea on a weighing balance and taking the readings thereof.

## **Germination (%)**

Fifteen cowpea seeds of each treatment were placed in petri dishes containing absorbent paper. They were replicated and arranged in a completely randomized design. Thereafter 2-3mls of water were added at regular intervals to keep the absorbent paper moist until germination occurred. Seed germination was assessed by counting the seeds with seed leaf and dividing by the total number of seeds in Petri plates expressed as percentage as reported by Liamngee *et al.* (2016). Thus,

Germination = 
$$\frac{\text{number of seeds germinated}}{\text{total number of seeds per plate}} \times 100....$$
 (iv)

## 2.6. Data Analysis

Data obtained from the study was analyzed using Analysis of Variance (ANOVA) and the Fishers least significant difference was used to separate the means at 5% level of significance.

#### 3. RESULTS

## 3.1. Preparing Callosobruchus maculatus Fab. Cultures for assessment tests

Female cowpea seed bruchids were differentiated from male seed bruchids by having an overall darker appearance in comparison to males which were brown in colour. The females had two large lateral dark patches midway along the elytra and smaller patches at the anterior and posterior ends while the males were much less distinctively marked. Also, the plate covering the end of the

abdomen was larger in females with smaller patches while in the males, the plate was smaller and lacked patches as shown in Plates 1 and 2.





Plate 1: FemaleCallosobruchus maculatus.

Plate 2: MaleCallosobruchus maculatus.

Eggs of the seed bruchid became visible on cowpea seeds (Plates 3 and 4) 3-4days after the adult male and female were introduced into the cowpea seeds. The eggs were small, grey and dome shaped with flat bases and were cemented to the surface of the seeds. Emerged progenies were visible 22-24 days after eggs were seen on white cowpea variety while for the brown cowpea variety; it took 24-25days for emerged progenies to become visible (Plates 5 and 6). Active progenies with flight abilities were visible 2days after emerged progenies were seen for both white and brown varieties of cowpea. The gestation period of *C. maculates* on both cowpea varieties was between three to four weeks as shown in Figure 1.



Plate 3: Eggs of Callosobruchus maculatusF. on white variety of Cowpea



Plate 4: Eggs of *Callosobruchus maculatus*F. on brown variety of Cowpea



Plate 5: Emerged Progeny of *Callosobruchus* maculatusfrom White cowpea variety

Plate 6: Emerged Progeny of *Callosobruchus*maculatusfrom brown cowpea

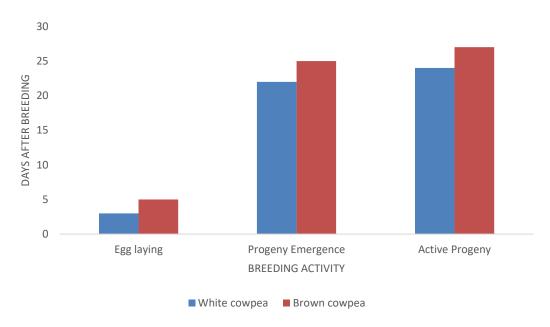


Figure 1: Visible stages in Cowpea Seed Bruchid Breeding

# 3.2. Effect of Neem leaf powder on weight of cowpea seeds during storage

The main and interaction effect of variety and rate of Neem leaf powder on the weight of cowpea seeds are shown in Tables 1 and 2 respectively. Results showed that the main effect of variety and rate as well as the interaction effect of variety and rate were significant on days 1, 7, 14, 21, and 28 (P<0.05). The interaction effect of variety and rate at 50g was significantly higher than the interaction of variety and rate at 0g and 25g respectively on days 1, 7, 14, 21 and 28. White variety of cowpea showed significantly higher weight compared to the brown variety on all days of storage. Also, 0g rate of Neem leaf powder showed significantly lower weight for both the main and interaction effect compared to the other rates on all days of storage.

Table 1: Main Effect of Variety and Rate on Weight of Cowpea seeds in Storage

Weight (g)						
1	7	14	21	28 (Days)		
40.52	37.47	34.33	31.39	27.07		
42.90	39.51	36.88	33.59	30.38		
1.56	1.50	1.92	2.54	3.76		
42.22	37.85	33.00	27.93	22.07		
41.07	38.23	36.47	34.07	32.35		
41.85	39.38	37.35	35.47	33.03		
0.58	1.23	1.57	2.07	3.07		
	40.52 42.90 1.56 42.22 41.07 41.85	1     7       40.52     37.47       42.90     39.51       1.56     1.50       42.22     37.85       41.07     38.23       41.85     39.38	1     7     14       40.52     37.47     34.33       42.90     39.51     36.88       1.56     1.50     1.92       42.22     37.85     33.00       41.07     38.23     36.47       41.85     39.38     37.35	1     7     14     21       40.52     37.47     34.33     31.39       42.90     39.51     36.88     33.59       1.56     1.50     1.92     2.54       42.22     37.85     33.00     27.93       41.07     38.23     36.47     34.07       41.85     39.38     37.35     35.47		

Table 2: Interaction Effect of Variety and Rates on Weight of Cowpea seeds in Storage

Variety	Rate	1	7	14	21	28 (Days)
Brown	0g	40.83	36.90	31.53	27.17	21.57
	25g	40.30	37.87	36.33	33.60	31.83
	50g	40.43	37.63	35.13	33.40	30.37
White	0g	43.60	38.80	34.47	28.70	22.57
	25g	41.83	38.60	36.60	34.53	32.87
	50g	43.27	41.13	39.57	37.53	35.70
LSD(0.05)		2.21	2.13	2.72	3.59	5.32

## 3.3. Effect of Neem leaf powder on germination of cowpea seeds during storage

The main and interaction effect of variety and rate of Neem leaf powder on germination are presented in Tables 3 and 4 respectively. Results obtained showed that the main and interaction effect of variety and rate of Neem leaf powder on germination of cowpea were statistically significant on all the days of storage. Higher germination was obtained for white variety of cowpea on days 1 (88.3), 7(88.3), 14 (73.3), 21(58.9) and 28(45.6) when compared to brown cowpea on days 1(77.8), 7(72.2), 14(62.2), 21(51.1) and 28(40.0) respectively and this was significant on all the days of storage. Also, significantly higher germination was obtained for 50g Neem leaf powder (91.7, 90.0, 81.7, 73.3 and 61.7) when compared to the other rates of application on all the days of storage respectively.

The interaction effect of variety and rate of Neem leaf powder on germination was significantly higher for variety treated with 50g Neem leaf powder than for varietal interactions at 25g and 0g rate of Neem leaf powder respectively. Control seeds showed lower germination and was significant compared to 25g and 50g respectively on all the days of storage.

Table 3: Main Effect of Variety and Rate on Germination of Cowpea Seeds in Storage

		Germination (n)							
	1	7	14	21	28 (Days)				
Variety									
Brown	77.80	72.20	62.20	51.10	40.00				

ANALYSIS	ARTICLE
----------	---------

White	83.30	83.30	73.30	58.90	45.60	
LSD(0.05)	7.26	7.26	7.84	9.38	10.27	
Rate						
0g	65.00	61.70	51.70	36.70	26.70	
25g	85.00	81.70	70.00	55.00	40.00	
50g	91.70	90.00	81.70	73.30	61.70	
LSD(0.05)	5.93	5.93	6.41	7.66	8.39	

Table 4: Interaction Effect of Variety and Rates on Germination of Cowpea Seeds in Storage

Germination (n)									
Rate	1	7	14	21	28 (Days)				
0g	63.30	56.70	50.00	33.30	26.70				
25g	83.30	76.70	63.30	53.30	36.70				
50g	86.70	83.30	73.30	66.70	56.70				
0g	66.70	66.70	53.30	40.00	26.70				
25g	86.70	86.70	76.70	56.70	43.30				
50g	96.70	96.70	90.00	80.00	66.70				
	10.27	10.27	11.09	13.26	14.53				
	0g 25g 50g 0g 25g	Rate     1       0g     63.30       25g     83.30       50g     86.70       0g     66.70       25g     86.70       50g     96.70	Rate         1         7           0g         63.30         56.70           25g         83.30         76.70           50g         86.70         83.30           0g         66.70         66.70           25g         86.70         86.70           50g         96.70         96.70	Germination (n)           Rate         1         7         14           0g         63.30         56.70         50.00           25g         83.30         76.70         63.30           50g         86.70         83.30         73.30           0g         66.70         66.70         53.30           25g         86.70         86.70         76.70           50g         96.70         96.70         90.00	Germination (n)           Rate         1         7         14         21           0g         63.30         56.70         50.00         33.30           25g         83.30         76.70         63.30         53.30           50g         86.70         83.30         73.30         66.70           0g         66.70         66.70         53.30         40.00           25g         86.70         86.70         76.70         56.70           50g         96.70         96.70         90.00         80.00				

# 3.4. Effect of Neem leaf powder on Insect population of Cowpea seeds during Storage

The main and interaction effect of variety and rate of application of Neem leaf powder on insect population was not significant on days 1 and 7 but was significant on days 14, 21 and 28 as shown in Tables 5 and 6 respectively. Significantly higher insect population was recorded for brown cowpea than white cowpea on days 14, 21 and 28 (15.50, 25.67 and 36.78 respectively). Also, 0g Neem leaf powder recorded significantly higher weevil population on days 7, 14, 21 and 28 (17.50, 28.22 and 40.50 respectively) compared to 25g and 50g respectively. Furthermore, significantly higher insect population was recorded for interaction of brown and white variety with 0g Neem leaf powder on days 14, 21 and 28 (18.67, 30.67 and 46.0) and (16.33, 26.00 and 35.00) respectively compared to the interaction effect of variety with 25g and 50g respectively.

Table 5: Main Effect of Variety and Rate on Insect population of Cowpea Seeds in Storage

		Insect Population (n)							
	1	7	14	21	28 (Days)				
Variety									
Brown	10.00	10.00	15.50	25.67	36.78				
White	10.00	10.00	14.44	21.22	28.33				
LSD(0.05)	NS	NS	0.93	1.45	4.39				
Rate									
0g	10.00	10.00	17.50	28.33	40.50				
25g	10.00	10.00	15.33	23.50	32.67				
50g	10.00	10.00	12.67	18.50	24.50				

LSD(0.05)	NS	NS	0.76	1.18	3.59
-----------	----	----	------	------	------

Table 6: Interaction Effect of Variety and Rates on Insect population of Cowpea Seeds in Storage

			Insect Population (n)					
Variety	Rate	1	7	14	21	28 (Days)		
Brown	0g	10.00	10.00	18.67	30.67	46.00		
	25g	10.00	10.00	16.00	26.33	35.67		
	50g	10.00	10.00	13.00	20.00	28.67		
White	0g	10.00	10.00	16.33	26.00	35.00		
	25g	10.00	10.00	14.67	20.67	29.67		
	50g	10.00	10.00	12.33	17.00	20.33		
LSD(0.05)		NS	NS	1.32	2.05	6.21		

## 3.5. Effect of Neem leaf Powder on Insect Mortality of Cowpea Seeds during Storage

The main effect of variety and rate of Neem leaf powder as well as the interaction effect of variety and rate of Neem leaf powder on insect mortality was not significant on day 1 but was significant on days 7, 14, 21 and 28 respectively as shown in Table 7. Brown variety of cowpea showed significantly higher insect mortality on day 7 (1.00) while white cowpea variety showed significantly higher insect mortality on days 14(2.33), 21(6.22) and 28(10.89) respectively. Rate of 50g of Neem leaf powder produced significantly higher insect mortality on days 14 (3.50), 21(8.17) and 28 (14.00) compared to 25g (2.00), (5.50), (9.67) and 0g (0.67), (2.83) and (4.00) respectively. Furthermore, significantly higher insect mortality was recorded for interaction of brown and white cowpea variety with 50gNeem leaf powder on days 14, 21 and 28 (3.33, 7.33, 12.00) and (3.67, 9.00, 16.00) respectively compared to the interaction effect of variety with 25g and 0g respectively on days 14, 21 and 28 as shown in Table 8.

Table 7: Main Effect of Variety and Rate on Insect mortality of Cowpea Seeds in Storage

		Insect Mortality(n)						
	1	7	14	21	28 (Days)			
Variety								
Brown	0.00	1.00	1.78	4.78	7.56			
White	0.00	0.00	2.33	6.22	10.89			
LSD(0.05)	NS	0.41	1.45	2.21	2.11			
Rate								
0g	0.00	0.00	0.67	2.83	4.00			
25g	0.00	0.66	2.00	5.50	9.67			
50g	0.00	0.83	3.50	8.17	14.00			
LSD(0.05)	NS	0.34	1.18	1.81	1.72			

Table 8: Interaction Effect of Variety and Rates on Insect Mortality of Cowpea Seeds in Storage

	Insect Mortality(n)							
Variety	Rate	1	7	14	21	28 (Days)		
Brown	0g	0.00	0.00	0.67	3.00	3.67		

ANALYSIS	ARTICLE						
	25g	0.00	1.33	1.33	4.00	7.00	
	50g	0.00	1.66	3.33	7.33	12.00	
White	0g	0.00	0.00	0.67	2.67	4.33	
	25g	0.00	0.00	2.67	7.00	12.33	
	50g	0.00	0.00	3.67	9.00	16.00	
LSD(0.05)		NS	0.59	2.05	3.13	2.99	_

# 3.6. Effect of Neem leaf powder on Cowpea seeds with holes

The main and interaction effect of variety and Neem leaf powder on seed with holes was not significant on day 1 but was significant on days 7, 14, 21 and 28 (P<0.05) as shown in Tables 9 and 10. Brown variety of cowpea showed significantly higher number of seeds with holes than white cowpea on days 7, 14, 21 and 28 (14.22, 23.11, 38.00 and 53.11) (12.78, 19.22, 25.67 and 37.00) respectively. Cowpea seeds not treated with Neem leaf powder (0g) also recorded significantly higher number of seeds with holes on days 7, 14, 21 and 28 (18.33, 29.33, 45.50 and 64.17) compared to rate of 25g and 50g respectively.

The interaction effect of variety and rate of application of Neem leaf powder on number of seeds with holes was significantly higher in brown and white cowpea variety with 0g Neem leaf powder on days 7, 14, 21 and 28 (18.33, 32.67, 56.67 and 75.67) and (18.33, 26.00, 34.33 and 52.67) respectively compared to all other treatment combinations as shown in Table 10.

Table 9: Main Effect of Variety and Rate on Seeds with holes of Cowpea in Storage

	Grain with Holes (n)						
	1	7	14	21	28 (Days)		
Variety							
Brown	0.00	14.22	23.11	38.00	53.11		
White	0.00	12.78	19.22	25.67	37.00		
LSD(0.05)	NS	2.17	3.16	4.52	3.93		
Rate							
0g	0.00	18.33	29.33	45.50	64.17		
25g	0.00	13.00	19.83	28.67	40.33		
50g	0.00	9.17	14.33	21.33	30.67		
LSD(0.05)	NS	01.77	2.58	3.69	3.21		

Table 10: Interaction Effect of Variety and Rates on Seed with holes of Cowpea in Storage

		Grains with Holes (n)				
Variety	Rate	1	7	14	21	28 (Days)
Brown	0g	0.00	18.33	32.67	56.67	75.67
	25g	0.00	13.67	21.00	32.67	47.00
	50g	0.00	10.67	15.67	24.67	36.67
White	0g	0.00	18.33	26.00	34.33	52.67
	25g	0.00	12.33	18.67	24.67	33.67
	50g	0.00	7.67	13.00	18.00	24.67
	50g	0.00	7.07	13.00	18.00	

LSD(0.05)	NS	3.08	4.47	6.40	5.56	
-----------	----	------	------	------	------	--

#### 3.7. Overall Effect of Neem leaf powder on Quality Parameters of Cowpea seeds in Storage

The overall main effect of variety and rate of Neem leaf powder as well as the overall interaction effect of variety and rate of Neem leaf powder on cowpea were significant for weight, germination, insect population, grain with holes and insect mortality as shown in Tables 11 and 12 respectively. White variety of cowpea showed significantly higher weight, germination and insect mortality (36.65g, 68.90% and 4.00 respectively) compared to the brown variety. Also, significantly higher weight, germination and insect mortality was recorded for cowpea seeds treated with 50g Neem leaf powder (39.44, 79.70 and 5.33 respectively) compared to 25g (36.46, 66.3 and 3.50) and 0g (32.71, 48.30 and 1.67) respectively while higher insect population and grain with holes was recorded for grains treated with 0g of Neem leaf powder (21.17 and 31.33) compared to 25g (19.33 and 20.33) and 50g (15.33 and 15.00) respectively as shown in Table 11.

The overall interaction effect of variety and rate of Neem leaf powder on cowpea showed that white and brown variety at 50g gave significantly higher weights (39.44 and 36.39), germination (86.00 and 73.30%) and insect mortality (6.00 and 4.67) compared to the other rates. Also, the interaction effect of both the white and brown varieties at 0g produced significantly higher insect population (19.33 and 26.00) and seed with holes (26.00 and 36.67) as shown in Table 12.

Table 11: Main Effect of Variety and Rate on quality parameters of Cowpea Seeds in Storage

	Quality Parameters						
	Weight	Germination	Insect	Seed with	Insect		
			Population	Holes	mortality		
Variety							
White	36.65	68.9	17.89	18.78	4.00		
Brown	34.73	60.7	19.67	25.67	3.00		
LSD(0.05)	1.214	5.99	2.596	1.937	0.906		
Rate							
0g	32.71	48.3	21.17	31.33	1.67		
25g	36.46	66.3	19.83	20.33	3.50		
50g	39.44	79.7	15.33	15.00	5.33		
LSD(0.05)	1.21	7.33	3.18	2.37	1.57		
L3D(0.03)	1.21	7.55	5.10	2.51	1.57		

Table 12: Interaction Effect of variety and rate on quality parameters of cowpea in Storage

				Insect	Seed with	
Variety	Rate	Weight	Germination	Population	holes	Insect mortalit
White	0g	33.63	50.7	19.33	26.00	1.67
	25g	36.89	70.0	20.00	18.00	4.33
	50g	39.44	86.0	14.33	12.33	6.00
Brown	0g	31.79	46.0	23.00	36.67	1.67
	25g	36.03	62.7	19.67	22.67	2.67
	50g	36.39	73.3	16.33	17.67	4.67
LSD(0.05)		1.83	10.37	4.49	3.35	1.57

#### 4. DISCUSSION

The efficacy of Neem leaf powder on cowpea seed bruchid (*C. maculatus* Fab.) was investigated. Duration from introduction of parent weevil to visibility of deposited eggs was 3-4days. This time frame is required for the parent seed bruchid to mate and for eggs to develop into larvae that migrate into the seeds. The time frame it took for emerged seed bruchid to become visible was higher for brown cowpea variety than for white cowpea. The differences observed may be due to genetic variations in cowpea varieties which are some of the factors that determine seed penetration by seed bruchid. A similar report was given by Lale (2002) who stated that genetic variation plays a vital role in the development of cowpea seed bruchids. Also, the short duration (24-48hours) for active flight by seed bruchids as observed in this study may be due to the short developmental phase of the young seed bruchid into mature adults once they emerge from the seeds. This is similar to the findings of the Center for Agriculture and Bioscience International (CABI) (2019) who reported 24-38hours for emerged cowpea seed bruchids to mature into adults.

During the study, there was a comparatively lower weight loss recorded in white cowpea variety than the brown variety. The lower weight loss recorded in the white variety of cowpea may be due to its lower insect infestation which implied lower feeding activity of the insects thereby preserving the weight of the seeds. The higher weight loss recorded in the brown variety may be due to the higher insect infestation in the variety which implied higher feeding activity of the insects leading to loss of weight in the seeds. Insects damage cowpea seeds by direct feeding. The insects feed on the endosperm resulting in loss of weight and quality of the grain. Also, the inherent properties of the brown cowpea seeds such as genetic factors, possible presence of biochemical content, such as tannins, phenols etc make them more palatable to seed bruchid larvae since the quantity of food consumed by the developing larvae of the insect is proportional to the amount of weight loss of the seed (Chijindu *et al.*, 2009). Also, there was a significantly lower weight reduction for cowpea treated with Neem leaf powder (25g and 50g) than the control (0g). This may be due to the presence of bioactive compounds such as alkaloids, tannins, flavonoids, saponins and azadirachtin in Neem which deter insect pests thereby reducing their feeding potentials and resulting in higher weight in treated seeds. This is in agreement with Okolo and lledun (2019) who reported least weight in control cowpea (untreated cowpea) when compared to those treated with other preservatives.

During the study, there was a significant difference in the germination of the different treatment combinations of cowpea. Seeds treated with Neem recorded higher germination than the control. This may be due to the fact that the Neem powders act as an antifeedant or modify the micro storage environment, preventing the insect from entering the seed to feed on the germ thereby increasing their germination percentage. The control seeds showed lower germination because the cowpea weevil was able to penetrate the seed and feed on the germ resulting in lower seed germination and less viability. The increased germination in treated seeds also implied that Neem leaf powder enhanced the germinability of seeds making them more productive in terms of improved viability. This according to Addul Kareem *et al.* (1989) is due to the ability of Neem to boost root and shoot growth indices in treated seeds.

During the study, it was observed that the interaction effect of cowpea variety and Neem rate at 50g showed significantly lower weevil population for treated seeds ofboth varieties compared to the control. This could be due to the ability of Neem leaf powder to arrest the development of the pest at certain stages such as the egg and larvae phases thereby lowering the number of progenies. This finding is in agreement with Lale (2002) who reported that Neem has about 12-15 complex constituents with repellant, insect growth regulatory, anti-feedant and pesticidal properties. The pesticidal effect is due to the presence of Azadirachtin which also functions as an insect repellant and sterilant (Ilesanmi and Gungula, 2010). The preservative ability of Neem leaf powder on cowpea as presented in this study also agrees with the findings of Neto et al. (2019) who stated that powder from Neem leaves have an insecticide action. Their study revealed that Neem powder has a repellant effect on weevils at all doses thereby affecting their population. This study is also in agreement with the report of Ilesanmi and Gungula (2010) who reported that Neem significantly suppress weevil population. Their study also validates the claim of increased suppression of weevil population at increased rates of application of Neem presented in this study. A possible reason for suppression of weevil population by application of Neem leaf powder according to Baba et al. (2014) might be due to suppression of the emergence of the F1 generation from late instar larvae of the insect.

During this study, it was observed that application of Neem leaf powder to cowpea seeds resulted in mortality of the seed bruchids. This may be due to the toxic/insecticidal effect of the Neem treatment causing interference in respiration resulting in suffocation and death of the insect. Azadirachtin in Neem is reported to have the ability to penetrate the cuticle of the seed bruchids thereby resulting in insect mortality (Ilesanmi and Gungula, 2010). This finding is also in agreement with that of Neto *et al.* (2019) who reported that powder from plant leaves promoted the death of weevils to a significant level. The pesticidal activity of Neem spam a wide spectrum, having repellent, phagodeterrent (antifeedant), insect growth regulator, fecundity, anti-ovipositional and fitness reducing properties on insects. Furthermore, findings from this study also showed that 50g rate of application of Neem leaf

powder resulted in higher mortality for the cowpea weevil *Callosobruchus maculates* F. compared to the 25g rate while control seeds without treatment experienced highest survival rate. This agrees with the report of Isinkaye and Ode (2018) who stated that the quantity of dosage applied has significant effect on weevil population. The finding of this study is also in agreement with Okolo and Iledun (2019) who reported least weevil mortality in control seeds.

During the study, it was observed that cowpea seeds treated with Neem leaf powder showed fewer seeds with holes compared to the untreated (control). This may be due to the toxic or repellant constituents of the Neem leaf powder which directly retarded all developmental stages and feeding behavior of the insect. This is in agreement with Abdulkareem *et al.* (1989) who reported reduction in number of grains with holes in treated seeds compared to untreated seeds. Cowpea weevil (*Callosobruchus maculatus* F.) is known to bore holes out through seeds when emerging from the late instar larvae. Also, feeding activity during progeny development results in holes in seeds. This act usually results in grain with holes and reduces quality attributes of grains to a great extent.

## 5. CONCLUSION

Neem leaf powder is a potent preservative for cowpea. Its ability to ensure quality attribute retention is validated in this study. Higher rates of application of Neem leaf powder from 50g is most effective if storage of the grains is the sole aim of the farmer. It is therefore a potent alternative for use in place of chemical preservatives dominant in the market today.

#### 5.1. Recommendations

Based on the findings of this study, the following recommendations are made:

- Farmers should adopt the use of Neem leaf powder for the preservation of cowpea in storage. This would help prevent the attendant dangers associated with use of chemical preservatives which are most common in our society today.
- Agricultural extension workers and postharvest physiologists should be funded with resources to train the local farmers on ways of
  utilizing organic preservatives such as Neem leaf powder. They should also be educated on methods of measuring powder rates
  for optimum preservative potentials.
- Further studies should be conducted in this aspect of science so as to ensure the availability of an array of literatures necessary for the development of a compendium of preservatives from organic origin.

#### **Funding**

This study has not received any external funding.

#### **Conflict of Interest**

The authors declare no conflicts of interests any matter related to this paper.

#### Data and materials availability

All related data have been presented in this paper.

#### Peer-review

External peer-review was done through double-blind method.

# REFERENCES AND NOTES

- 1. Abbey, B.W. (2006). Bridging the protein gap with what you have. University of Port *Harcourt Inaugural Lecture Series, No.* 48, Port Harcourt, Nigeria. Pp.25.
- Abdul Kareem, R.C. Saxena, M.E.M. Boncodin, V. Krishnasamy, and D.V.Seshu. (1989). Neem as seed treatment for rice before sowing: effects on two homopterous insects and seedling vigor. J. Econ. Entomol, 82: 1219-1223.
- Abulude, F.O., Ogunkoya, M.O. and Ogunleye, R.F. (2007). Storage properties of oils of two Nigeria oil seeds *Jatropha curcas* (Physic nut) and *Helianthus annus* (Sunflower). *America Journal of Food Technology*, 2 (3): 207-211
- Baba G.O., Erhabor T.A., Sulaiman Y.D., Ayanrinde F.A., Majolagbe M.O., Fadoyin A.S. and Musa K. (2014). Biopesticidal Effect of Neem Plant Products (Azadirachtaindica) on the Mortality of Late Instar Larvae and Emergence of F1 generation of Dermestid Beetle (Dermestes macalatus Degeer) on Dried fish (Clarias spp.). Global Journal of Biology, Agriculture and health Sciences, 3(1):314-321.
- Center for Agriculture and Bioscience International CABI (2019). Undated a. CABI Compendium: Status as determined by CABI editor. Wallingford, UK: CABI (2019).

- Chijindu, E.N., Okonkwo, N.J., Umeweni, N.T. and Ndidi, S.U. (2009). Susceptibility of Cowpea varieties (*Vigna unguiculata*) (L) Walp to Infestation by *Callosobruchus maculatus* (F) (Coleoptera: Bruchidae). *Journal of Applied Sciences*, 12(1):8369-8379.
- Ilesanmi, J.O. and Gungula, D.T. (2010). Preservation of Cowpea (Vigna unguiculata) Grains against Cowpea Bruchids (Callosobrochus macalatus) Using Neem and Moringa seed oils. International Journal of Agronomy, 235280:108.
- 8. Isinkaye, O.D. and Oke, D.O. (2018). The Effect of Neem and Garlic as Pesticide in Cowpea Storage. *Global Scientific Journal*, 6(1):209-217.
- 9. Lale, N.E.(2002). Bio-activity and Limitation against wide spread use of Neem products for the management of insect pests. *Nigerian J. Applied Biol.*, 3: 115-124.
- Liamngee, K., Zakki, Y.H. and Ogidi, P.E. (2016). The Efficacy of Aloe vera Coating on Post-Harvest Shelf life and Quality of Tomato Fruits during Storage. Asian Research Journal of Agriculture, 8(4):1-9.
- Neto, E.P., Andrade, B.A., Costa, E.M., Maracaja, P.B., Santos, A.B., Santos, J.L. and Pimenta, T.A. (2019). Effect of Neem Powder (*Azadirachta indica*) on the control of Cowpea weevils inn Cowpea beans. *Journal of Experimental Agriculture International*, 30(2):1-7.
- Njonjo, M.W., Muthomi, J.W. and Kutima, H.L. (2014).
   Production practices, postharvest handling, and quality of cowpea seeds used by farmers in Makueni and Taita Taveta counties in Kenya. *International journal of Agronomy*,1-12.
- 13. Nigerian Meteorological Association (2017). Makurdi, Nigeria: Climate Information.
- 14. Okolo, E.T. and Iledun, O.C. (2019). Insecticidal Effects of Neem (Azadirachta indica) Extracts obtained from Leaves and Seeds on Pests of Cowpea (Vigna unguiculata). Sumerians Journal of Agriculture and Veterinary, 2(4):20-28.
- Sallam,M.N.(2011). Insect damage: Postharvest operations (Postharvest Compendium). http://www.fao.org/in-action/inpho/crop-compendium/pests/en/.
- 16. Tello, V., Diaz, L., Sanchez, M. (2013). Side effects of the natural pesticide Spinosad (GF-120 formulation) on Eretmocerus paulistus (Hymenoptera: Aphelinidae), a parasitoid of the white fly Aleurothrixus floccosus (Hemiptera: Aleyrodidae), under laboratory conditions. Cien. Innovation in Agriculture, 40(2):407-417.
- Zakki, Y.H., Liamngee, K., Owoicho, A.L. and Agatsa, T.D. (2017). Effect of Neem leaf powder on postharvest shelf life and Quality of Tomato Fruits in Storage. *International Journal of Development and Sustainability*, 6(10):1334-1349.